

**EFFECTS OF BEE BREAD SUPPLEMENTATION  
ON ENDURANCE RUNNING PERFORMANCE,  
BONE METABOLISM MARKERS, ANTIOXIDANT  
STATUS AND SELECTED PHYSIOLOGICAL  
PARAMETERS IN ATHLETES**

**FADZEL WONG CHEE PING**

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**EFFECTS OF BEE BREAD SUPPLEMENTATION ON ENDURANCE  
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ANTIOXIDANT STATUS AND SELECTED PHYSIOLOGICAL PARAMETERS  
IN ATHLETES**

**by**

**FADZEL WONG CHEE PING**

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**KESAN PENGAMBILAN ROTI LEBAH TERHADAP PRESTASI DAYA  
TAHAN LARIAN, PENANDA METABOLISMA TULANG, STATUS  
ANTIOKSIDAN DAN PARAMETER FISIOLOGI TERPILIH DALAM  
KALANGAN ATLET**

**ABSTRAK**

**PENGENALAN:** Data saintifik mengenai kesan ergogenik pengambilan produk lebah semasa pra dan pasca senaman adalah sedikit. **MATLAMAT:** Kajian ini terdiri daripada dua fasa. Fasa pertama kajian menyelidik kesan pengambilan 8 minggu roti lebah pra senaman terhadap prestasi daya tahan larian, kuasa anaerobik, kekuatan dan kuasa otot isokinetik, status antioksidan, penanda metabolisme tulang dan parameter fisiologi terpilih dalam kalangan atlet. Fasa kedua kajian menyelidik kesan pengambilan roti lebah semasa pemulihan selepas senaman terhadap prestasi daya tahan larian dan parameter fisiologi terpilih dalam kalangan atlet. **KAEDAH: Fasa pertama-** Dua belas atlet lelaki (umur:  $24.0 \pm 1.8$  tahun; IJT:  $22.3 \pm 1.3$  kg.m<sup>-2</sup>; VO<sub>2max</sub>:  $52.0 \pm 2.8$  mL.kg<sup>-1</sup>.min<sup>-1</sup>) telah dipilih untuk menyertai kajian ini yang rawak terkawal dan plasebo tertutup bersilang. Peserta mengambil roti lebah dalam dos 20 g.hari<sup>-1</sup> atau placebo selama 8 minggu sebelum ujian eksperimen. Bagi mengukur prestasi larian daya tahan, peserta dikehendaki berlari pada 60% daripada VO<sub>2max</sub> masing-masing di atas treadmill bermotor selama 90 minit dan diikuti segera dengan 20 minit prestasi larian masa. Denyutan jantung, suhu timpanik dan kadar aras kepenatan (RPE) direkodkan dalam

selang masa 10 minit. Pengambilan oksigen diukur dalam selang masa 20 minit. Sampel darah dikumpulkan dalam selang masa 20 minit semasa larian eksperimen untuk menentukan plasma glukosa, insulin, laktat, kortisol, asid lemak, hemoglobin, hematokrit dan perubahan isipadu plasma. Sampel darah juga dikutip pada pra-suplemen, pra-senaman, pasca-senaman serta-merta dan 24 jam pasca senaman untuk menentukan jumlah status antioksidan (TAS), nisbah GSH: GSSG, F<sub>2</sub>-Isoprostane dan penanda metabolisme tulang (serum jumlah kalsium, fosforus, fosfatase alkaline, osteokalsin dan telopeptide C-terminal jenis 1 kolagen (1CTP). Kuasa anaerobik, dan kekuatan dan kuasa otot isokinetik peserta diukur sebelum dan selepas 8 minggu tempoh eksperimen. Stastitik dianalisis dengan menggunakan Anova ulangan pengukuran dan ujian pasangan t. **Fasa kedua-** Dua belas atlet (umur:  $22.3 \pm 2.9$  tahun; IJT:  $22.8 \pm 2.1$  kg.m<sup>-2</sup>; VO<sub>2max</sub>:  $50.0 \pm 6.4$  mL.kg<sup>-1</sup>.min<sup>-1</sup>) dipilih untuk menyertai kajian ini yang rawak terkawal dan plasebo tertutup bersilang. Kajian ini terbahagi kepada dua peringkat pemulihan. Pada hari eksperimen dalam pemulihan pertama, peserta berlari di atas treadmill bermotor selama 90 minit pada 60% VO<sub>2max</sub> dan diikuti dengan rehat selama 4 jam. Dalam proses pemulihan pertama ini, peserta makan 30 g.jam<sup>-1</sup> roti lebah atau plasebo bersama dengan air dengan kadar 150% kehilangan berat badan. Denyutan jantung dan suhu timpanik diukur pada setiap 20 minit semasa pemulihan 4 jam. Sampel darah diambil pada setiap 30 minit semasa pemulihan 4 jam untuk mengukur plasma glukosa, kortisol, insulin, laktat, hemoglobin, hematocrit dan perubahan isipadu plasma. Sampel darah juga diambil sebelum senaman, selepas 90 minit senaman, selepas 4 jam pemulihan, selepas prestasi larian masa dan selepas 24 jam senaman untuk mengukur jumlah antioksidan status, nisbah GSH: GSSG, F<sub>2</sub>-Isoprostane dan kreatin kinase. Selepas rehat, peserta berlari 20 minit prestasi larian masa di atas treadmill bermotor.

Dalam proses pemulihan kedua, peserta sambung makan roti lebah dalam dos 40 g.hari<sup>-1</sup> atau plasebo selama 3 hari. Selepas 3 hari pemulihan, peserta berlari di atas treadmill bermotor selama 90 minit pada 60%  $VO_{2max}$  dan seterusnya diikuti dengan 20 minit prestasi larian masa. Denyutan jantung, suhu timpanik dan penanda aras kepenatan diukur dalam setiap selang masa 10 minit. Pengambilan oksigen diukur dalam setiap selang masa 20 minit semasa senaman. Sampel darah diambil pada setiap 20 minit semasa larian eksperimen untuk mengukur plasma glukosa, laktat, kortisol, insulin, hemoglobin, hematokrit dan perubahan isipadu plasma. Sampel darah juga diambil sebelum suplemen, sebelum senaman, selepas senaman dan 24 jam selepas senaman untuk mengukur jumlah antioksidan status, nisbah GSH: GSSG, F<sub>2</sub>-Isoprostane dan kreatin kinase. Stastitik dianalisis dengan menggunakan Anova ulangan pengukuran dan ujian pasangan t. **KEPUTUSAN: Fasa pertama-** Jarak yang diliputi dalam roti lebah eksperimen adalah lebih jauh signifikan berbanding dengan plasebo eksperimen dalam ujian larian masa. TAS dan nisbah GSH: GSSG adalah lebih tinggi manakala F<sub>2</sub>-Isoprostane adalah lebih rendah dalam roti lebah eksperimen berbanding dengan plasebo eksperimen selepas pengambilan roti lebah selama 8 minggu, serta-merta pasca senaman dan 24 jam selepas senaman. Walau bagaimanapun, denyutan jantung, suhu timpanik, kadar aras kepenatan, pengambilan oksigen, glukosa, kortisol, insulin dan laktat dalam eksperimen roti lebah adalah tidak beza secara signifikan berbanding dengan eksperimen plasebo. Mengenai penanda metabolisme tulang, tiada perbezaan yang signifikan dalam serum jumlah kalsium, fosforus, fosfatase alkali, osteokalsin dan telopeptide C-terminal jenis 1 kolagen (1CTP) antara eksperimen plasebo dan eksperimen roti lebah. Mengenai kuasa anaerobik, tiada perbezaan yang signifikan dalam kuasa min, kuasa puncak, kapasiti anaerobik dan kuasa anaerobik antara eksperimen plasebo dan eksperimen roti

lebah pada pra dan pasca ujian suplemen. Mengenai kekuatan dan kuasa otot isokinetik, isokinetik lutut kanan ekstensi puncak tork dan kuasa purata pada  $180^{\circ} \cdot s^{-1}$ , dan isokinetik lutut kanan fleksi puncak tork dan kuasa purata pada  $180^{\circ} \cdot s^{-1}$  adalah signifikan lebih tinggi di pasca ujian suplemen berbanding dengan pra ujian suplemen dalam eksperimen roti lebah. **Fasa kedua-** Dalam pemulihan pertama selama 4 jam, jarak yang diliputi oleh eksperimen roti lebah adalah lebih jauh signifikan daripada eksperimen plasebo dalam ujian larian masa. Plasma glukosa, insulin dan nisbah GSH: GSSG adalah lebih tinggi manakala  $F_2$ -Isoprotane adalah lebih rendah dalam eksperimen roti lebah berbanding dengan eksperimen plasebo semasa pemulihan selama 4 jam. Walau bagaimanapun, denyutan jantung, suhu timpanik, tanda aras kepenatan, pengambilan oksigen, hemoglobin, hematokrit, perubahan isipadu plasma, kortisol, laktat dan kreatin kinase di dalam eksperimen roti lebah adalah tidak signifikan beza daripada eksperimen plasebo. Dalam pemulihan kedua selama 3 hari, tiada perbezaan yang signifikan dalam jarak yang diliputi dalam ujian larian masa diantara eksperimen roti lebah dengan eksperimen plasebo. Denyutan jantung, suhu timpanik, tanda aras kepenatan, pengambilan oksigen, hemoglobin, hematokrit, penukaran isipadu plasma, glukosa, laktat, insulin, kortisol, jumlah status antioksidan, nisbah GSH: GSSG dan  $F_2$ -Isoprostane di dalam eksperimen roti lebah adalah tidak signifikan beza daripada eksperimen plasebo. **KESIMPULAN: Fasa pertama-** Pengambilan suplemen roti lebah selama 8 minggu dalam dos  $20 \text{ g} \cdot \text{hari}^{-1}$  memberi kesan ergogenik terhadap prestasi larian daya tahan. **Fasa kedua-** Pengambilan suplemen roti lebah dalam dos  $30 \text{ g} \cdot \text{jam}^{-1}$  dalam masa 4 jam pemulihan menunjukkan kesan yang positif terhadap prestasi seterusnya larian daya tahan. Walau bagaimanapun, kesan ergogenik ini terhadap prestasi larian



daya tahan tidak terbukti ekoran daripada pengambilan suplemen roti lebah pada dos 40 g.hari<sup>-1</sup> selama 3 hari semasa pemulihan.

**EFFECTS OF BEE BREAD SUPPLEMENTATION ON ENDURANCE  
RUNNING PERFORMANCE, BONE METABOLISM MARKERS,  
ANTIOXIDANT STATUS AND SELECTED PHYSIOLOGICAL  
PARAMETERS IN ATHLETES**

**ABSTRACT**

**INTRODUCTION:** Scientific data on the ergogenic effects of bee products consumed at pre exercise and post exercise on sports performance is scanty. **PURPOSE:** This present study consisted of two phases. The first phase study investigated effects of 8-week bee bread supplementation at pre exercise on endurance running performance, anaerobic power, isokinetic muscular strength and power, antioxidant status and bone metabolism markers in athletes. The second phase study investigated the effects of bee bread supplementation at post exercise, i.e. during recovery on subsequent running performance and physiological parameters in athletes. **METHODS: First phase-** Twelve male athletes (age:  $24.0 \pm 1.8$  years old; BMI:  $22.3 \pm 1.3$  kg.m<sup>-2</sup>; VO<sub>2max</sub>:  $52.0 \pm 2.8$  mL.kg<sup>-1</sup>.min<sup>-1</sup>) were recruited in this randomised double blind; placebo-controlled crossover study. Participants consumed either bee bread at a dosage of 20 g.d<sup>-1</sup> or placebo for 8 weeks prior to the experimental trial. For the measurement of endurance running performance, participants were required to run at 60% of their respective VO<sub>2max</sub> on a treadmill for 90 minutes and immediately followed by a 20 minutes running

time trial performance on a motorised treadmill. Heart rate, tympanic temperature and rate of perceived exertion (RPE) were recorded at intervals of 10 minutes. Oxygen uptake was measured at intervals of 20 minutes. Blood samples were collected at interval of 20 minutes during trial to determine plasma glucose, insulin, lactate, cortisol, free fatty acid, hemoglobin, hematocrit and plasma volume changes. Blood samples were also collected at pre supplementation, pre exercise, immediate post exercise and 24 hours post exercise to determine total antioxidant status (TAS), reduced and oxidised glutathione (GSH: GSSG) ratio, F<sub>2</sub>-Isoprostanes and bone metabolism markers (serum total calcium, phosphorus, alkaline phosphatase, osteocalcin and C-terminal telopeptide of type 1 collagen (1CTP). Participants' anaerobic power and isokinetic muscular strength and power were measured before and after 8 weeks of experimental period. Statistical analyses were performed using ANOVA with repeated measures and paired t test. **Second phase-** Twelve athletes (age:  $22.3 \pm 2.9$  years old; BMI:  $22.8 \pm 2.1$  kg.m<sup>-2</sup>; VO<sub>2max</sub>:  $50.0 \pm 6.4$  mL.kg<sup>-1</sup>.min<sup>-1</sup>) were recruited in this randomised double blind; placebo-controlled crossover study. This study was divided into two stages of recovery. In the first stage of recovery, participants ran at 60% of their respective VO<sub>2max</sub> on a treadmill for 90 minutes and then rested for 4 hours. During this first recovery period, participants consumed either bee bread at a dosage of 30 g.h<sup>-1</sup> or placebo along with water equivalent to 150% of body weight loss. Heart rate and tympanic temperature were measured at intervals of 20 minutes during the 4 hours recovery. Blood samples were collected at intervals of 30 minutes during the 4 hours recovery to determine plasma glucose, cortisol, insulin, lactate, hemoglobin, hematocrit and plasma volume changes. Blood samples were also collected at pre exercise, immediate post 90 minutes running, immediate post 240 minutes recovery, immediate post time trial and 24 hours

post exercise to determine total antioxidant status, GSH: GSSG ratio, F<sub>2</sub>-Isoprostane and creatine kinase. After which, participants performed 20 minutes running time trial performance on a motorised treadmill. During the second stage of recovery after the run, participants continued consuming either bee bread at a dosage of 40 g.day<sup>-1</sup> for 3 days or continued consuming placebo. After 3 days of recovery, participants ran at 60% of their respective VO<sub>2max</sub> on a treadmill for 90 minutes and immediately followed by a 20 minutes running time trial performance. Heart rate, tympanic temperature and rate of perceived exertion (RPE) were recorded at intervals of 10 minutes. Oxygen uptake was measured at intervals of 20 minutes. Blood samples were collected at interval of 20 minutes during trial to determine plasma glucose, lactate, cortisol, insulin, hemoglobin, hematocrit and plasma volume changes. Blood samples were also collected at pre supplementation, pre exercise, immediate post exercise and 24 hours post exercise to determine total antioxidant status, GSH: GSSG ratio, F<sub>2</sub>-Isoprostane and creatine kinase. Statistical analyses were performed using ANOVA with repeated measures and paired t test. **RESULTS: First phase-** Distance covered in the running time trial for bee bread trial was significantly longer in comparison with the placebo trial. TAS and GSH:GSSG ratio were significantly higher, whereas F<sub>2</sub>-Isoprostane was significantly lower in the bee bread trial in comparison with placebo trial following supplementation of bee bread for 8 weeks, immediate post exercise and 24 hours post exercise. However, heart rate, tympanic temperature, rate of perceived exertion, oxygen uptake, glucose, cortisol, insulin and lactate in the bee bread trial were not significantly different from the placebo trial. Regarding bone metabolism markers, there were no significant different in serum total calcium, phosphorus, alkaline phosphatase, osteocalcin and C-terminal telopeptide of type 1 collagen (ICTP) between placebo and bee bread trial. Regarding anaerobic

power, there were no significant difference in mean power, peak power, anaerobic capacity and anaerobic power between placebo and bee bread trial at pre and post supplementation test. Regarding isokinetic muscular strength and power, isokinetic right knee extension peak torque and average power at  $180^{\circ} \cdot s^{-1}$ , and isokinetic right knee flexion peak torque and average power at  $180^{\circ} \cdot s^{-1}$  were significantly higher at post supplementation test compared to pre supplementation test in the bee bread trial. **Second phase-** In the first stage of 4 hours recovery, distance covered in the bee bread trial was significantly longer than placebo trial in the running time trial performance. Plasma glucose, insulin and GSH: GSSG ratio was significantly higher, whereas F<sub>2</sub>-Isoprostane was significantly lower in the bee bread trial in comparison with placebo trial during 4 hours recovery. However, heart rate, tympanic temperature, rate of perceived exertion, oxygen uptake, hemoglobin, hematocrit, plasma volume changes, cortisol, lactate and creatine kinase in the bee bread trial were not significantly different compared to the placebo trials. In the second stage of 3 days recovery, there was no significant difference in the distance covered in the bee bread trial compared to the placebo trial. Heart rate, tympanic temperature, rate of perceived exertion, oxygen uptake, hemoglobin, hematocrit, plasma volume changes, glucose, lactate, insulin cortisol, total antioxidant status, GSH: GSSG ratio and F<sub>2</sub>-Isoprostane in the bee bread trial were not significantly different compared to the placebo trial. **CONCLUSION: First phase-** Supplementation of bee bread for 8 weeks at a dosage of 20 g daily elicited ergogenic effects on running time trial performance. **Second phase-** Supplementation of bee bread at a dosage of 30 g.h<sup>-1</sup> during 4 hours recovery seems to indicate beneficial effects on subsequent endurance running performance. However, this ergogenic effect on subsequent

endurance running performance was not evident following supplementation of bee bread at a dosage of 40 g.day<sup>-1</sup> for 3 days during recovery.

## **CHAPTER 1**

### **INTRODUCTION**

Nutritional ergogenic aids are substances which can enhance athletic performance by influencing physiological and psychological processes (Brouns, 2002). The term “ergogenic aids” is derived from the Greek words, whereby the word “ergon” means “work” and “gennan” means “to produce” (Antonio and Stout, 2001). According to William (2010), nutritional strategies are the common types of ergogenic aids used by sportspersons to enhance their exercise performance.

Sportspersons usually take supplements before and after a sports competition to make sure they have adequate nutrition, maximize their energy storage and enhance their sports performance (Manore and Thompson, 2000). Supplements may enhance endurance performance by increasing the available glucose and free fatty acid during exercise, increasing body glycogen storage in muscle and liver, and increasing antioxidant status (Antonio and Stout, 2001). The increasing availability of adenosine triphosphate (ATP) in the working muscles will augment aerobic and anaerobic sports performance (Krause *et al.*, 2010). Endurance athletes such as marathon runners and triathlons are known to use supplements to enhance their performance. (Manore and Thompson, 2000).

Recovery is a challenge for sportspersons who are undertaking two or more sessions each day, training for prolonged periods, or competing in a programme that

involves multiple events. A number of studies indicated that recovery of endurance capacity was enhanced by intake of dietary carbohydrate and protein during recovery period (Betts *et al.*, 2008; Skillen *et al.*, 2008; Howarth *et al.*, 2009; Alghannam., 2011; Cathcart *et al.*, 2011; Ferguson-Stegall *et al.*, 2011b; Hara *et al.*, 2011). Blom *et al.* (1987) reported that consuming carbohydrate ( $0.18\text{-}0.35\text{ g.kg}^{-1}.\text{kh}^{-1}$ ) can increase 150% glycogen storage but further intake of carbohydrate ( $0.35\text{-}0.7\text{g.kg}^{-1}.\text{h}^{-1}$ ) did not enhance the glycogen re-synthesis storage. Furthermore, Parkin *et al.* (1997) also reported that consuming of carbohydrate would not provide further enhancement of glycogen storage for a long recovery period between 8 - 24 hours as long as total carbohydrate intake was adequate.

There are two glycogen pools namely macroglycogen and proglycogen, which are involved in the glycogen synthesis and utilization. Macroglycogen pool will reach glycogen super compensation after 2-3 days, while proglycogen will reach maximum after 2-4 hours (Adoma and Graham, 1998). Numerous studies have reported that supplementation of carbohydrate and protein may improve glycogen repletion (Zawadzki *et al.*, 1992; Van Loon *et al.*, 2000; Ivy *et al.*, 2002; Williams *et al.*, 2003); protein balance (Koopman *et al.*, 2004); and sports performance (Niles *et al.*, 2001; Williams *et al.*, 2003; Romano-Ely *et al.*, 2006). Protein exerted a synergistic effect on the insulin release when administered separately or in combination with a carbohydrate load (Zawadzki *et al.*, 1992; Van Hall *et al.*, 2000). Insulin stimulates muscle glucose uptake and activation of glycogen synthesis (Ivy, 1998). Recently, it was reported that ingestion of  $0.04\text{g kg}^{-1}.\text{h}^{-1}$  protein with less carbohydrate during recovery period will stimulate glycogen resynthesis, muscle protein synthesis, inhibit protein breakdown and allow net muscle protein accretion

(Beelen *et al.*, 2010). Betts and Williams (2010) reported that by adding  $\geq 0.3 \text{ g.kg}^{-1} \cdot \text{h}^{-1}$  of protein to a carbohydrate supplement results in a synergistic increase in insulin secretion and accelerate muscle glycogen resynthesis during recovery period. Kreider and Campbell (2009) highlighted that sportsperson needs more protein ( $1.4 - 2.0 \text{ g.kg}^{-1} \cdot \text{day}^{-1}$ ) compared to the sedentary ones to repair damaged cell and tissues, synthesis hormones, promote growth and variety of metabolic activities.

It has been reported by Niles *et al.* (2001) that supplementation of carbohydrate and protein following glycogen depleting exercise facilitated a greater rate of muscle glycogen resynthesis than a carbohydrate supplementation alone, and could speed up the recovery process and improved endurance performance during a second bout of exercise performed on the same day. In addition, Ivy *et al.* (2002) also reported that carbohydrate and protein supplementation is more effective for rapid replenishment of muscle glycogen after exercise than a supplementation of carbohydrate alone of equal caloric content. The rate of glycogen storage during the carbohydrate and protein supplementation was found to be 38% higher than during the trial in which only carbohydrate was consumed in a study by Zawadzki *et al.* (1992). Ferguson-Stegall *et al.* (2011a) also reported that supplementing carbohydrate and protein post-exercise improves aerobic power ( $\text{VO}_{2\text{max}}$ ) more effectively than supplementation of carbohydrate alone. Similarly, several previous studies also reported that consumption of carbohydrate and protein for 4 hours after exercising would restore depleted muscle glycogen stores and muscle re-synthesis (Coyle, 1991; Robergs, 1991; Levenhagen *et al.*, 2001; Williams *et al.*, 2003; Betts *et al.*, 2008). Additionally, Murphy and Miller (2010) indicated that consumption of



protein and carbohydrate beverage for 4 hours during recovery period after aerobic exercise increased whole protein turnover than carbohydrate beverage.

Ren *et al.* (2011) reported that Grass carp protein supplementation at dosage of 1 kg of body weight<sup>-1</sup>.day<sup>-1</sup> for 4 weeks significantly improved time trial swimming performance in mice. Shing *et al.* (2006) indicated that sportsperson supplemented with Bovine colostrum protein concentrate at a dosage of 10g.day<sup>-1</sup> for 8 weeks significantly had shorter time to complete 40 km time trial performance in comparison with placebo. Matsumoto *et al.* (2009) observed that ingestion of branched chain amino acid (BCAA) at a dosage of BCAA drink (0.04% BCAA, 4% CHO: 1500 mL/day) for 6 days increased lactate threshold and may be effective to increase endurance exercise capacity.

Bee bread is made of pollen, which has been gathered by bees and mixed with its own digestive enzymes, packed into pellets and preserved with a tiny bit of honey and bee wax. This mixture undergoes different chemical processes due to the action of different enzymes, micro-organisms, moisture and temperature at 35 - 36 °C (Nagai *et al.*, 2004). Bee bread is rich in glucose, essential amino acids, unsaturated fatty acids, multi vitamins and minerals (Nagai *et al.*, 2001). Bee bread has antioxidant properties (Nagai *et al.*, 2001; Nagai *et al.*, 2005) and antibacterial activity (Baltrusaityle *et al.*, 2007; Abouda *et al.*, 2011). Latest study indicated that bee bread enriched with unsaturated free fatty acid and has a higher  $\omega$ -3 fatty acid /  $\omega$ -6 fatty acid ratio. Bee bread fatty acid ratio is more suitable for human diet than many plant oils and it was able to restore optimal ratio of unsaturated fatty acids  $\omega$ -6:

$\omega$ -3 in the diet to be 1: 2, as compared to the modern diet, in which this ratio was unbalance at 1: 15 - 20 (Ceksteryte and Jansen, 2012).

Other related studies reported that bee products such as bee pollen has anti-inflammatory (Maruyama *et al.*, 2010), antitumor (Yang *et al.*, 2007) and anti-allergic (Medeiros *et al.*, 2008) properties. It was also well documented that another bee product, honey, has antioxidant (Cowan, 1999; Chen *et al.*, 2000; Gheldof *et al.*, 2002; Al-Mamary *et al.*, 2002; Aljadi and Kamaruddin, 2004), anti-inflammatory (Cooper *et al.*, 2001; Zaharil *et al.*, 2011), antimicrobial (Lusby *et al.*, 2005; Sherlock *et al.*, 2010), antimutagenic (Wang *et al.*, 2002), and antitumor (Swellam *et al.*, 2003; Tomasin and Gomez-Marcondes, 2011) properties.

There are limited studies on effect of bee products supplementation on sports performance. For instances, Earnest *et al.* (2004) reported that supplementation of honey (low glycaemic index) at a dosage of 15g every 16 km during a simulated 64-km cycling time trial was significantly faster to complete time trial in comparison with placebo trial. Shukri *et al.* (2011) found that ingestion of 500 ml of honey drink one hour before trial and 3 ml. kg body weight<sup>-1</sup> of cool honey drink every 20 minutes during the running trial was as good as a sports drink in improving running time trial performance and has similar effects on blood glucose in a hot environment. Nechaeva (2009) reported that ingestion of 10 g of pollen for 15 days among Russian sport female students significantly improved viso-motoric reaction in comparison with the placebo trial. However, Abbey and Rankin (2009) reported that acute supplementation of honey beverage at a dosage of 1g.kg body weight<sup>-1</sup> before and

during soccer- stimulation test did not significantly improve progressive shuttle-run (PSR) test to exhaustion in comparison with the placebo trial.

Bee bread possesses remarkable antioxidant and free radical scavenging abilities (Nagai *et al.*, 2001; Nagai *et al.*, 2005). Antioxidant is a substance that helps to reduce the severity of oxidative stress. It has been well-documented that free radicals increase with increased intensity and duration of exercise (Manore and Thompson, 2000). Raised body temperature has also been shown to increase free radicals production in athletes (Jakson, 1995). This oxidative stress may limit the exercise performance because the free radicals can cause cell damage and promote muscular fatigue. Exercise is postulated to generate free radicals by production of lactic acid that can convert a weakly damaging free radical (superoxide) into a strongly damaging one (hydroxyl) and also due to inflammatory responses to muscle damage (Sen, 1995; Dekkers *et al.*, 1996).

Bee products have been used traditionally for treatment of various diseases and its therapeutic value has partly been related to its antioxidant properties. In a study done by Mohamed *et al.* (2011), honey supplementation at a dosage of 1.2 g.kg body weight<sup>-1</sup> honey for 13 weeks has antioxidant protective effects against cigarette smoke induced testicular damage in male rats. Lily (2010) also has found that daily intake of honey at 20 mg.day<sup>-1</sup> for 4 months is safe to be used and has similar effect on bone densitometry when compared with hormone replacement therapy.

Tavafzadeh *et al.* (2011) indicated that combination of jumping exercise and honey supplementation (dosage of 1 g.kg of body weight day<sup>-1</sup> for 8 weeks) may

elicit beneficial effects on tibia and femur bone generally when compared to either jumping exercise or honey supplementation alone in young female rats. Rahim *et al.* (2011) reported that combination of aerobic dance exercise and honey supplementation (20 g of honey diluted in 300 mL of plain water for 8 weeks) elicited more beneficial effects on bone health and immune function generally compared to aerobic dance exercise or honey supplementation alone in sedentary women.

To our knowledge, no study has been carried out to investigate the possible beneficial effects of bee bread supplementation at pre exercise on running time trial performance, anaerobic power, isokinetic muscular strength and power, bone metabolism markers and antioxidant status in athletes. Furthermore, there were also no scientific studies that have been carried out to investigate the effects of bee bread supplementation during recovery on subsequent running performance. Therefore, the aim of this study was to investigate effects bee bread supplementation on endurance running performance, anaerobic power, isokinetic muscular strength and power, bone metabolism markers, antioxidant status and recovery on subsequent running performance.

## **1.1 Objectives of the Study**

This study was carried out in two phases namely phase one study and phase two study.

### **1.1.1 Objectives of Phase One Study**

1. To determine the effects of daily bee bread supplementation for 8 weeks on running time trial performance and selected physiological parameters such as plasma glucose, plasma insulin, plasma cortisol, plasma lactate, plasma free fatty acid, plasma hemoglobin, plasma hematocrit, serum total antioxidant status, serum reduced glutathione: oxidised glutathione (GSH: GSSG) ratio, and serum F<sub>2</sub>-Isoprostane in athletes.
2. To determine the effects of daily bee bread supplementation for 8 weeks on anaerobic power and isokinetic muscular strength and power in athletes.
3. To determine the effects of daily bee bread supplementation for 8 weeks on bone metabolism markers and antioxidant status in athletes.

### **1.1.2 Objectives of Phase Two Study**

1. To investigate the effects of bee bread supplementation during 4 hours post exercise recovery on subsequent running time trial performance.
2. To investigate the effects of bee bread supplementation after 3 days post exercise recovery on subsequent running time trial performance.

3. To investigate the effects of bee bread supplementation during 4 hours post exercise recovery on selected physiological parameters such as plasma glucose, plasma insulin, plasma cortisol, plasma lactate, plasma free fatty acid, plasma hemoglobin, plasma hematocrit, serum total antioxidant status, serum reduced glutathione: oxidised glutathione (GSH: GSSG) ratio, serum F<sub>2</sub>-Isoprostanes and serum creatine kinase during running time trial performance.
4. To investigate the effects of bee bread supplementation after three days post exercise recovery on selected physiological parameters such as plasma glucose, plasma insulin, plasma cortisol, plasma lactate, plasma free fatty acid, plasma hemoglobin, plasma hematocrit, serum total antioxidant status, serum reduced glutathione: oxidised glutathione (GSH: GSSG) ratio, serum F<sub>2</sub>-Isoprostanes and serum creatine kinase during running time trial performance.

## **1.2 Research Hypotheses**

### **1.2.1 Alternative Hypotheses for Phase One Study**

*H<sub>A1</sub>*: There are significant differences in running time trial performance and selected physiological parameters following 8 weeks supplementation of bee bread compared to the placebo trial.

*H<sub>A2</sub>*: There are significant differences in anaerobic power and isokinetic muscular strength and power following 8 weeks supplementation of bee bread compared to the placebo trial.

$H_{A3}$ : There are significant differences in bone metabolism markers and antioxidant status following 8 weeks supplementation of bee bread compared to the placebo trial.

### 1.2.2 Alternative Hypotheses for Phase Two Study

$H_{A1}$ : There is a significant difference in running time trial performance with bee bread supplementation during 4 hours post exercise recovery compared to the placebo trial.

$H_{A2}$ : There is a significant difference in running time trial performance with bee bread supplementation after 3 days post exercise recovery compared to the placebo trial.

$H_{A3}$ : There are significant differences in selected physiological parameters during running time trial performance with bee bread supplementation during 4 hours post exercise recovery compared to the placebo trial.

$H_{A4}$ : There are significant differences in selected physiological parameters during running time trial performance with bee bread supplementation after three days post exercise recovery compared to the placebo trial.

### **1.3 Significance of the Study**

To our knowledge, this is the first study carried out to investigate effects of bee bread supplementation consumed pre exercise on running time trial performance, anaerobic power, isokinetic muscular strength and power, bone metabolism markers, antioxidant status and also bee bread supplementation consumption during recovery on subsequent running performance. A few studies have reported ergogenic effects of honey on sports performance but no studies have investigated the ergogenic effects of bee bread on sports performance. Furthermore, some researchers have reported the antioxidant properties of bee bread but currently, there is no scientific data on the potential effectiveness of its antioxidant properties on sports performance. Thus, a scientific study is warranted to investigate the potential ergogenic effects of bee bread on sports performance. If the present study showed any positive effects of bee bread supplementation, it can then be recommended to the sportsperson to consume bee bread before and after a sports competition for enhancing their sports performance. The findings of the present study will be beneficial for a sportsperson who prepares for a sports tournament, undertaking two or more sessions each day, training for prolonged periods, or competing in a tournament that involves multiple events. Besides that, bee bread can also be recommended to the sportsperson and public for enhancing their health status due to its nutritious' content and antioxidant property.



## CHAPTER 2

### LITERATURE REVIEW

#### 2.1 Bee Products and Bee Bread

Bee products have been used thousands of years ago as a supplement and in traditional medicine. It is believed that bee products are the best supplement to human health but it is lacking in solid scientific evidences to prove its efficacy. Thus, it has received scientists' attention to conduct scientific research to test this supposition. Bee products include honey, propolis, royal jelly, bee pollen and bee bread. To date, several scientific experiments on bee products have been tested on their antioxidant (Orhan *et al.*, 1999; Chen *et al.*, 2000; Nagai *et al.*, 2001; Havsteen, 2002; Al-Mamary *et al.*, 2002; Gheldof *et al.*, 2002; Aljadi and Kamaruddin, 2004; Nagai *et al.*, 2004; Lusby *et al.*, 2005), anti-inflammatory (Cooper *et al.*, 2001; Havsteen, 2002; Al-Waili *et al.*, 2003; Zaharil *et al.*, 2011; Candiracci *et al.*, 2012; Leong *et al.*, 2012), anti-microbial (Lusby *et al.*, 2005; Orsi *et al.*, 2005; Baltrusaityle *et al.*, 2006; Duaete *et al.*, 2006; Sherlock *et al.*, 2010; Abouda *et al.*, 2011) and anti-tumour activities (Swellam *et al.*, 2003; Wang *et al.*, 2002; Barbaric *et al.*, 2011; Borges *et al.*, 2011; Tomasin *et al.*, 2011; Wu *et al.*, 2011; Da Silva Frozza *et al.*, 2012; Saxena *et al.*, 2012). However, there is limited information of ergogenic effects of bee products on exercise performance. Additionally, there is no scientific research conducted on the effectiveness of bee bread supplementation on exercise performance.

Bee bread is made of pollen, which has been gathered by bees and mixed with its own digestive enzymes, carried back to the hive, packed into pellets and preserved with a tiny bit of honey and bee wax. This mixture undergoes different chemical processes by the action of different enzymes, micro-organisms, moisture and temperature (35-36 °C), and bee bread is formed after 2 weeks (Nagai *et al.*, 2004). Besides containing carbohydrates, bee bread is rich in essential amino acids, unsaturated fatty acids, multi vitamins and minerals (Nagai *et al.*, 2001; Nagai *et al.*, 2004).

The nutrient composition of bee bread differs slightly from bee pollen. It has higher content of vitamin K and low pH compared to bee pollen. The higher prebiotic activity of bee bread causes a good preservation of bee bread due to the inhibition of the growing of moulds and microorganism. It does not require any specific preservation with chemicals for a longer storage. Honey mixed with bee bread contributes aromatic components (Ceksteryte and Jansen, 2012) and this mixture was used together with other medicaments for treating arthritis (Ceksteryte *et al.*, 2008).

Bee bread is composed of well-balanced proteins containing all essential amino acids that the body is unable to biosynthesis (Nagai *et al.*, 2001). It also contains vitamins such as B1, B2, E, nicotinic acid, folic acid, pantothenic acid, pigments and other biologically active compounds, enzymes such as saccharase, amylase, phosphatase, flavonoids, carotenoids, and hormones. Vitamin B contained in bee bread is important for regulation of metabolism and functioning of nervous system. Bee bread has over 25 different micro and macro minerals such as iron,

calcium, phosphorus, potassium, copper, zinc, selenium, magnesium, and also active substances with antibacterial action (Ceksteryte *et al.*, 2008).

In a recent study carried out by Ceksteryte and Jansen (2012), it was found that bee bread is enriched with unsaturated free fatty acid and has a high  $\omega$ -3 fatty acid /  $\omega$ -6 fatty acid ratio. This bee bread fatty acid ratio is more suitable for human diet than many plant oils, and it was able to restore the optimal ratio of unsaturated fatty acids  $\omega$ -6:  $\omega$ -3 in the diet to be 1: 2, which in the modern diet, this ratio was unbalance at 1: 15 - 20 (Ceksteryte and Jansen, 2012). The human body is unable to synthesis polyunsaturated fatty acids such as  $\omega$ -3 and  $\omega$ -6, and they are needed to be consumed via food. The  $\omega$ -3 fatty acids in human diet are  $\alpha$ -linolenic (ALA), docosahexaenoic (DHA) and eicosapentaenoic (EPA) acids (Bierenbaum *et al.*, 1993). Docosahexaenoic (DHA) and eicosapentaenoic (EPA) acids also possess cardio protective properties through reducing blood cholesterol and exerting an anti-arrhythmic, anti-thrombotic and anti-inflammatory effects (Ceksteryte *et al.*, 2008).

Bee bread consumption can lower cholesterol level, and the higher quantities of magnesium and calcium make it to be efficient in cases of heart diseases and maintains a high level of energy (Nagai *et al.*, 2004). The lactic acid bacteria existing in bee bread are probiotics to give strength to the body, support the digestive tract and effectively increase nutrient absorption (Ceksteryte and Jansen, 2012). Katz *et al.* (1977) mentioned that 5 species of bacteria belonging to the genus *Bacillus* were identified in bee bread. *Bacillus subtilis* is a metabolically active bacterium producing antibiotics and methyl-branched fatty acids. Wojcicki *et al.* (2012) reported that bee bread may lower serum cholesterol level, inhibit platelet

aggregation and promote vessel wall production for prostacyclin, in which prostacyclin is both a potent inhibitor of platelet aggregation and a powerful vasodilator (Seppanen *et al.*, 1989).

### **2.1.1 Effects of Bee Products Supplementation on Antioxidant Status**

Numerous studies indicated that free oxygen species are the major causes of cardiovascular diseases (Hertog *et al.*, 1993; Rakha *et al.*, 2008), cancer (Ginter, 1995; Dragan *et al.*, 2007; Hassan *et al.*, 2012) diabetes (Erejuwa *et al.*, 2010), impaired wound healing (Efem, 1988; Subrahmanyam, 1991; Wana, 1997), cataracts (Gerster, 1989), and gastrointestinal disorders (Haffeejee, & Moosa 1985; Smirnov, 1994; Ali, 1995; Ladas *et al.*, 1995). Reactive oxygen species (ROS) and reactive nitrogen species (RNS) such as hydroxyl radical, superoxide, hydrogen peroxide, nitric oxide and peroxynitrite can damage lipids, proteins, and DNA in cells (Orrenius *et al.*, 2007). Antioxidants will scavenge the free radical from damaging the cell. To date, the exact antioxidant mechanism is unknown, but the proposed mechanism is through hydrogen donation, free radical sequestration, metallic ion chelation, as substrates for hydroxyl and superoxide radical actions (Van Acker *et al.*, 1996).

Reactive oxygen species and reactive nitrogen species are also related to the muscle damage, fatigue and decrease exercise performance (Wolinsky and Driskell, 2008). It has been well-documented that free radicals increase with increased intensity and duration of exercise (Manore and Thompson, 2000). Raised body temperature has also been shown to increase free radicals production in athletes

(Powers and Howley, 2004). This oxidative stress may limit the exercise performance because the free radicals can cause cell damage and promote muscular fatigue. Exercise is postulated to generate free radicals by the production of lactic acid that can convert a weakly damaging free radical (superoxide) into a strongly damaging one (hydroxyl) and due to inflammatory responses to muscle damage (Sen, 1995; Dekkers *et al.*, 1996).

Natural antioxidants can be phenolic compounds such as flavonoid, and phenolic acids, as well as nitrogen compounds such as alkaloids, chlorophyll derivatives, amino acids and amines (Larson, 1988; Hall and Cuppet, 1997). Natural food usually contains antioxidants such as vitamin C, Vitamin E and carotenoids which can scavenge free radicals (Stampfer and Rimm, 1995). Honey contains antioxidant components such as vitamins C and E (Crane, 1975), phenolic compounds (Ferrerres *et al.*, 1992; Havsteen, 2002; Al-Mamary *et al.*, 2002; Lusby *et al.*, 2005), catalase (Schepartz, 1966) and peroxidase (Ioyrish, 1974). It is well documented that bee product such as honey (Cowan 1999; Chen *et al.*, 2000; Havsteen, 2002; Al-Mamary *et al.*, 2002; Gheldof *et al.*, 2002; Aljadi and Kamaruddin, 2004; Lusby *et al.*, 2005), propolis (Orhan *et al.*, 1999) and bee bread (Nagai *et al.*, 2001; Nagai *et al.*, 2004; Audisio *et al.*, 2005; Stanciu *et al.*, 2007) exhibited a strong antioxidant activity.

Bee products have been used traditionally for treatment of various diseases and its therapeutic value has partly been related to its antioxidant properties. It is also reported that the antitumor effect of honey, a bee product, may be attributed to its antioxidant activity (Hassan *et al.*, 2012). In an animal study done by Mohamed *et al.*

(2011), 32 adult male rats were randomly divided into 4 groups: control group, honey treated, cigarette smoke exposed and honey treated plus cigarette exposed. Honey supplementation was given at a dosage of 1.2g, kg<sup>-1</sup> body weight honey for 13 weeks. This study found that honey has antioxidant protective effects against cigarette smoke induced testicular damage in male rats.

Bee bread extracts have high antioxidant abilities and scavenging abilities against free radical and reactive oxygen species (ROS) such as superoxide anion radical and hydroxyl radical (Nagai *et al.*, 2001). Stanciu *et al.* (2009) found that bee bread has antioxidant components such as phenolic acids and flavonoids. Phenolic compounds are considered as possible protective agent in reducing damage in human body from free radical and retard the progress of lipid peroxidation (Gulcin *et al.*, 2003). Interestingly, scientific studies indicated that phenolic compounds are more effective antioxidants in comparison with vitamins C and E (Vinson *et al.*, 1995; Cao *et al.*, 1997). Phenolic compounds are very efficient scavenger of peroxy radicals because of their molecule structures that have an aromatic ring with hydroxyl groups containing mobile hydrogen (Halliwell, 1990; Aruoma, 1994). Phenolic compounds also can reduce and chelate ferric ion and catalyse lipid peroxidation (Gazzani *et al.*, 1998).

To date, it is well documented that bee bread is an antioxidant agent but no scientific study has been carried up to investigate the effectiveness of its antioxidant properties on exercise performance. Based on the previous studies, it is evident that antioxidant agent is important to the sportsperson for enhancing their exercise

performance. Thus, a scientific study is warranted to investigate the ergogenic effects of bee bread on exercise performance.

### **2.1.2 Effects of Bee Products Supplementation on Bone**

Bone metabolism or bone remodelling is a process of bone formation or bone resorption in human. Bone resorption is referring to the mature bone tissue being removed from the skeleton, while bone formation is referring to new bone tissue being formed. Remodelling responds also have functional demands on the mechanical loading. To date, several studies related to bee product, i.e. honey reported that honey supplementation combined with jumping exercise in animals (Tavafzadeh *et al.*, 2011; Mosavat *et al.*, 2014); honey supplementation combined with aerobic dance in humans (Ooi *et al.*, 2011) and honey supplementation alone in animals (Ariefdjohan *et al.*, 2006; Chepulis and Starkey, 2008;) were beneficial for enhancing bone health. However, to dates no scientific study has been carried up to investigate the effectiveness of bee bread on bone metabolism markers.

Chepulis and Starkey (2008) reported that honey feeding alone for 52 weeks increased bone mineral density in rats in comparison with sucrose and sugar free diet feeding. Ariefdjohan *et al.* (2006) reported that ingestion of honey for 8 weeks increase calcium absorption after acute feeding in growing rats. It is reported higher calcium retention and calcium absorption in the group of rat given 10% of honey than 5% of honey and control group. However, there were no significant differences in femur density and femur bone mineral between the groups. In an animal study

done by Tavafzadeh *et al.* (2011), forty eight 12-week old female rats were divided into four groups: control group, honey supplementation group, jumping exercise group without supplementation group, and combined jumping exercise and honey supplementation group. Oral honey supplementation was given to the rats at dosage of 1 g/kg of body weight/day for 8 weeks. Jumping exercise consisted of 40 jumps per day for 5 days per week at the height of 40 cm. This study found that combination of jumping exercise and honey supplementation may elicit beneficial effects on lower extremity bone properties and bone metabolism when compared to either jumping exercise or honey supplementation alone or control group in young female rats.

In a human study by Ooi *et al.* (2011), it was found that combination of aerobic dance exercise and honey supplementation i.e. 20 g of honey diluted in 300 mL of plain water for 8 weeks elicited more beneficial effects on bone turnover markers generally compared to aerobic dance exercise or honey supplementation alone in sedentary young female. A recent study by Mosavat *et al.* (2014) investigated honey supplementation combined with different jumping exercise intensities on bone mass and serum bone metabolism markers. This study indicated that supplementation of honey with 80 jumps per day, 5 days per week for 8 weeks elicited greatest beneficial effects on tibial and femoral mass, bone metabolism markers such as serum total calcium and alkaline phosphatase concentrations. This study concluded that high intensity jumping exercise combined with honey supplementation has more discernable beneficial effects on bone mass and bone metabolism.



### 2.1.3 Effects of Bee Products Supplementation on Exercise Performance

There is limited information of scientific research on effectiveness of bee products supplementation on exercise performance. To date, there are only a few related studies on effect of bee products supplementation on exercise performance (Earnest *et al.*, 2004; Abbey and Rankin, 2009; Ahmad *et al.*, 2011, Shukri *et al.*, 2011, Samsani *et al.*, 2012). However, to our knowledge, no scientific study has been carried up to investigate the ergogenic aids effects of bee bread supplementation on exercise performance.

Earnest *et al.* (2004) investigated the effect of low and high glycemic (GI) carbohydrate feedings during a simulated 64-km cycling time trial. Subjects ingested 15 g of low GI (honey) or high GI (dextrose) or placebo every 16 km during cycling time trial. This study reported that time to complete 64 km cycling time trial for honey trial =  $128 \pm 4$  minutes; dextrose trial =  $128 \text{ minutes} \pm 4$  minutes; placebo trial =  $131 \text{ minutes} \pm 4$  minutes. The authors concluded that supplementation of low GI (honey) at a dosage of 15 g every 16 km during a simulated 64-km cycling time trial (TT) significantly reduced the time to complete 64 km cycling time trial performance in comparison with placebo trial.

In another honey supplementation study, Shukri *et al.* (2011) investigated the effects of honey supplementation pre and during exercise on physiological responses and running time trial performance in the hot environment. Subjects were requested to run at 60%  $\text{VO}_{2\text{max}}$  for 60 minutes and immediately followed by 20 minutes time trial performance in a hot ( $31^{\circ}\text{C}$ ) and 70% relative humidity.

Subjects ingested 500 ml of honey drink one hour before trial and 3ml per kg of body weight of cool honey every 20 minutes during the running trial. This study found that distance covered in the honey trial was  $3.34 \pm 0.3$  km and sports drink was  $3.25 \pm 0.4$ . There was no significant difference in distance covered in the 20 minutes time trial performance between the two trials. There were also no significant difference in tympanic temperature, heart rate, rate of perceived exertion, oxygen uptake, haemoglobin concentration, haematocrit concentration, plasma volume changes, plasma glucose, insulin cortisol and total antioxidant status and body weight changes between the two trials. The authors concluded that honey drink was as good as commercial sports drink in improving running time trial performance and has similar effects on blood glucose in a hot environment.

Ahmad *et al.* (2011) investigated the effects of sodium enriched honey drink supplementation during rehydration after exercise on physiological changes and subsequent running performance in a hot and humid environment. Subjects were required to run on a treadmill for 65%  $\text{VO}_{2\text{max}}$  for 60 minutes. After that, subjects were required to rest for 2 hours and they were given either plain water, honey drink or sodium enriched honey drink with equivalent to 150% of body weight lost at 0 min, 30 min and 60 min. Then subjects were required to perform 20 minutes time trial performance. This study reported that running distance covered by the subjects in the 20 minutes time trial performance was  $3.42 \pm 0.35$  km in honey trial,  $3.39 \pm 0.39$  km in sodium enriched honey drink, and  $3.12 \pm 0.34$  km in plain water trials. The distance covered in 20 minutes time trial performance for honey trial and sodium enriched honey trial elicited +9.6 % and 8.7% in comparison with plain water respectively. There were significant differences in plasma glucose, insulin,

cortisol during rehydration and time trial running phase in honey trial and sodium enriched honey drink trial compared to plain water trial respectively ( $p < 0.05$ ). There were no significant differences on tympanic temperature, heart rate, oxygen uptake, perceived rate of exertion, plasma volume changes, haematocrit concentration and body weight changes for all trials. Honey drink seems to have elicited similar effects as sodium enriched honey drink in running performance and physiological parameters in a hot and humid environment. This study indicated that Acacia honey and sodium enriched Acacia honey drink elicited more beneficial effects than plain water and both of them can be recommended as an ergogenic aid for rehydration in a hot and humid environment.

Samsani *et al.* (2012) also carried up a study to compare the effects of Acacia honey drink supplementation and sports drink during rehydration after exercise on physiological parameters and subsequent running performance in the heat. Subjects were required to run on a treadmill for 70%  $\text{VO}_{2\text{max}}$  for 60 minutes in the heat ( $31^{\circ}\text{C}$ , 70% relative humidity). After that, subjects were required to rest for 2 hours and they were given either plain water, honey drink or sports drink which was equivalent to 150% of body weight loss at 0 min, 30 min and 60 min respectively. Then subjects were required to perform 20 minutes time trial performance. The distance covered in 20 minutes for water trial was  $3019 \pm 246.3$  m, honey trial was  $3268 \pm 215.9$  m and sports drink trial was  $3230 \pm 263.8$  m, respectively. Distance covered in the honey trial was significantly longer (8.24%) in comparison with plain water trial ( $p < 0.05$ ). However, there was no statistical difference between sports drink and water trial. Plasma glucose and free fatty acid in Acacia honey trial and sport drink trial were significantly higher in comparison with water trial,

respectively, during time trial running performance. There were no significant differences in body weight changes, oxygen uptake, heart rate, rate of perceived exertion, tympanic temperature, room temperature, relative humidity, haematocrit, plasma volume changes and plasma cortisol in all trials. This study indicated that Acacia honey drink elicited similar beneficial effects as sports drink and it can be used as an ergogenic aid for rehydration purposes in a hot and humid environment.

Abbey and Rankin (2009) investigated the effect of a honey-sweetened beverage, commercial sports drink and placebo on exercise performance. Subjects consumed beverage before and during halftime for a total of 1.0 g per kg of body weight of weight of carbohydrate for honey trial and sports drink trial. Performance measures included 5 sets of a high-intensity run, agility and ball-shooting tests followed by a final progressive shuttle-run test to exhaustion. This study reported that no significant effect of the interventions was observed for any performance measures. This study indicated that acute ingestion of honey drink before and during a soccer-simulation test did not significantly improve results of progressive shuttle-run test to exhaustion in comparison with placebo trial.

The evidence from the above mentioned previous studies indicating the ergogenic aids of bee products (Earnest *et al.*, 2004; Ahmad *et al.*, 2011, Shukri *et al.*, 2011, Samsani *et al.*, 2012), it is postulated that bee bread supplementation pre and post exercise will improve the endurance performance. To date, no scientific study has been carried out to investigate the possible beneficial effects of short term supplementation of bee bread on endurance running performance, anaerobic power, isokinetic muscular strength and power, bone metabolism markers and antioxidant

status. Although, some studies have reported on antioxidant properties of bee bread but there is no scientific study to investigate the possible beneficial ergogenic effect of its antioxidant properties on exercise performance. There are also scientific studies on the effect of honey on bone metabolism markers but to date no study has been reported on the effects of bee bread on this context. This scientific study is warranted to ascertain the purported ergogenic aid effects of bee bread supplementation on endurance running performance, anaerobic power, isokinetic muscular strength and power, bone metabolism markers, antioxidant status and recovery on subsequent exercise performance.

## 2.2 Carbohydrate Metabolism and Exercise

Carbohydrate is a macronutrient and acts as primary source of fuel for the working muscles during exercise. Carbohydrate is an organic compound consisting carbon, hydrogen and oxygen ( $\text{CH}_2\text{O}$ )  $n$ , where  $n$  equals three to seven carbon atoms. In the human body, carbohydrate is found as blood sugar and glycogen storage in the muscle and liver. Carbohydrate is divided or categorised into monosaccharide, disaccharides, oligosaccharides and polysaccharides (Table 2.1).

**Table 2.1** Four categories of carbohydrate in food

Monosaccharide	Glucose, Galactose, Fructose
Disaccharides	Lactose, Maltose, Sucrose
Oligosaccharides	Fructo-oligosaccharides, Raffinose, Stachyose
Polysaccharides	Alga polysaccharides, b-Glucans, Cellulose, Fructans, Pectins, Hemicelluloses